Research Article

Phosphatase Regenerating Liver-3 and E-Cadherin Expression in Epithelial Ovarian Cancer

Ekspresi Phosphatase Regenerating Liver-3 dan E-Cadherin pada Kanker Ovarium Epitel

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Abstract

Objective: To determine the expression of Phosphatase Regenerating Liver-3 (PRL-3) and E-Cadherin in the epithelial ovarian cancer on various stages and differentiation grades.

Method: This was a cross-sectional study design conducted at Obstetrics and Gynecology Department of several teaching hospitals, Faculty of Medicine Universitas Hasanuddin from January to June 2015. The expression of PRL-3 and E-cadherin was assessed immunohistochemically in 40 patients with epithelial ovarian cancer including 15 patients in early stage and 25 patients in advanced stage. We used the Fisher's exact test with the significance of $p<0.05$ for the statistical analysis. The Spearman correlation test was used to analyze the correlation between PRL-3 and E-cadherin expression.

Result: Our study showed no statistically significant difference of PRL-3 expression to the stage and the differentiation degree of epithelial ovarian cancer ($p>0.05$). The significant difference was found in the expression of E-cadherin whereas the high expression was shown at early stage than advanced stage ($p<0.05$). There was no significance difference between degree of differentiation and E-cadherin expression ($p>0.05$). This study also pointed out no correlation between the expression of PRL-3 and E-cadherin in epithelial ovarian cancer ($p>0.05$).

Conclusion: PRL-3 overexpression does not decrease E-cadherin expression in epithelial ovarian cancer.

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Keywords: E-cadherin, epithelial ovarian cancer, PRL-3

Abstrak

***Tujuan**: Menilai ekspresi Phophastase Regenerating Liver-3 (PRL-3) dan E-cadherin dihubungkan dengan stadium dan derajat differensiasi kanker ovarium epitel.*

***Metode**: Penelitian potong lintang yang dilakukan pada beberapa rumah sakit pendidikan pada Departemen Obstetri dan Ginekologi Fakultas Kedokteran Universitas Hasanuddin dari Januari sampai Juni 2015. Penilaian ekspresi PRL-3 dan E-cadherin secara imunohistokimia dilakukan pada 40 pasien kanker ovarium epitel (15 pasien stadium awal, 25 pasien stadium lanjut). Data dianalisis menggunakan uji Fisher's exact dengan tingkat kemaknaan $p<0,05$. Uji korelasi Spearman digunakan untuk menilai korelasi antara ekpresi PRL-3 dan E-cadherin pada kanker ovarium epitel.*

***Hasil**: Penelitian kami menunjukkan tidak ada perbedaan bermakna ekspresi PRL-3 terhadap stadium dan derajat diferensiasi kanker ovarium epitel ($p>0,05$). Perbedaan bermakna ditemukan pada ekspresi E-cadherin di mana ekspresi lebih tinggi pada stadium awal dibandingkan stadium lanjut ($p<0,05$), sedangkan tidak ada perbedaan bermakna antara tingkat diferensiasi dan ekspresi E-cadherin ($p>0,05$). Penelitian ini juga menemukan ekspresi PRL-3 tidak berkorelasi dengan E-cadherin pada kanker ovarium epitel ($p>0,05$).*

***Kesimpulan**: Ekpresi berlebihan PRL-3 tidak menurunkan ekspresi E-cadherin pada kanker ovarium epitel.*

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***Kata kunci**: E-cadherin, kanker ovarium epitel, PRL-3*

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INTRODUCTION

Ovarian cancer is the sixth common disease which contributes to higher death incidence in women worlwide.[1] More than 200.000 women died because of ovarian cancer, especially in women living in low income among developed and developing countries.[2] The highest rate of death is located in sub-Saharan Africa, including South Africa (40/100.000 people). In Africa, most of the patients with ovarian cancer are diagnosed in advanced stage disease (59.3% is diagnosed in stage III). Whereas the decrease of ovarian cancer incidence and mortality is documented in developed countries, such as USA, Canada, and Scandinavia; conversely, this documentation is not completed in developing countries due to the lack of efficiency from the screening programme.[3]

In recent years, there is the development of molecular biology in ovarian carcinoma to discover number of therapeutic targets and new molecular

prognostic factors, such as phosphatase regenerating liver-3 (PRL-3) and E-cadherin. Phosphatase Regenerating Liver-3 is a part of the protein tyrosine phosphatase that has a role in regulating the intracellular growth pathway. Some studies suggest that PRL phosphatase, especially PRL-3, plays important function in growth, proliferation, cell motility and invasion regulation.[4] The PRL-3 also has a role in the process of carcinogenesis. Specifically, PRL-3 is involved in the reconstruction of the cytoskeleton, adhesion and cancer cell cycle regulation, also epithelial-mesenchymal transition. Through this mechanism, PRL-3 is involved in invasion, migration, metastasis, and angiogenesis. Higher expression of PRL-3 is associated with tumor progression and severity of the disease. The over expression of PRL-3 is varied based on the type of tumor; many studies showed that tumor metastases both in lymph nodes and other organs would increase the PRL-3 expression extremely.[5]

E-cadherin (E-cad), the cell adhesion molecule plays an important role in maintaining tissue integrity. Impaired function of E-cadherin has often been associated with tumor formation and invasion in vivo and in vitro.[6] E-cadherin is expressed on epithelial cells and it is important for epithelial cells differentiation and cells adhesion during embryogenesis.[7] Abnormal expression of E-cadherin is associated with higher invasiveness and poor differentiation from several types of epithelial carcinomas.[8]

According to the theory above, we have not ever reported the association between PRL-3 and E-cadherin in epithelial ovarian cancer in Indonesia, especially Makassar. Therefore, this study aims to determine the role of PRL-3 and E-cadherin in epithelial ovarian cancer progressivity. Further more, we would like to assess the relationship between the expression of PRL-3 and E-cadherin through clinicopathological factors, as potential target for epithelial ovarian cancer therapy.

METHODS

This cross-sectional study was conducted from January to June 2015. Tumor tissues were collected from 40 patients who had been undergone surgery for epithelial ovarian carcinoma at Obstetrics and Gynecology Department of several teaching hospitals, Faculty of Medicine Universitas Hasanuddin. The analysis of immunohistochemical was performed at Anatomical Pathology Laboratory, Department of Anatomical Pathology Faculty of Medicine Universitas Hasanuddin and Pathology Laboratory Dr. Wahidin Sudirohusodo Hospital Makassar. We got the written informed consent from the patients before performing this study.

Tumor tissues taken during surgery were fixed in buffered and paraffin-embedded formalin. The tissue blocks were cut using a microtome into 4-μm-thickness sections on silanized glasses. After that, the tissue sections were immersed in hot water at 40°C and dried on hot plate at 60°C for 1 hour. We would store the sections at room temperature.

We performed the immunohistochemical analysis using streptavidin-biotin-peroxidase labeled streptavidin-biotin (Dako, Carpinteria, USA). Before staining, the tissue sections were deparaffinized in xylenes for 15 minutes. Then, the tissue sections were washed with dH_2O two times for 5 minutes each and incubated with Phosphate-buffered saline (PBS) for 5 minutes. In order to exhibit antigen, the tissue sections were heated in an autoclave for 15 minutes in citrate buffer. The tissue sections were cooled at room temperature for 1 hour and after drying, they were demarcated using a pap pen. After that, we repeated the washing with dH_2O for 5 minutes and PBS for 5 minutes before incubating them into 0.3% hydrogen peroxidase for 15 minutes. After endogenous peroxidase blocked, the tissue sections were incubated with blocking solution for 30 minutes to block avidin contained in the sections. The tissue sections were incubated overnight at -4°C with primary PRL-3 antibody (Santa Cruz) diluted 1 : 100 and E-cadherin monoclonal antibody (Biocare) diluted 1 : 100. The tissue sections would be washed three times with dH_2O before incubating with secondary antibodies and streptavidin for 30 minutes. The 3,3'-diamino-benzidine tetrahydrocloride was used for staining approximately 10 minutes to obtain the staining reaction that could be detected by microscopic examination. Subsequently, the sections were stained with hematoxylin eosin to clarify the nucleus of a cell for 30 seconds and they were washed with running water for 5 minutes. The tissue sections would be dehydrated in alcohol gradually from 70%, 80%, 90% to 100% for every 2 minutes. Then, the sections were dipped in xylene for 5 minutes and covered with cover slip after malinol application. The staining degree of PRL-3 and E-cadherin expression could be seen

from the percentage of cell group stained. The percentage was obtained from the sum of positive cells in the entire field of tissue sections view examination using a light microscope.

Immunohistochemical scoring system for protein E-cadherin and PRL on epithelial ovarian cancer uses three parameters consisting of intensity of expression, the percentage of positive cells, and expression patterns. The expression of E-cadherin is as follows: grade 1 = score 1-3; grade 2 = score 4-6; grade 3 = score 7-9; grade 4 = score ≥10. The grade 1 and 2 are classified as low level of expression; while, grade 3 and 4 are classified as high level of expression.

The Fisher's exact test was used to analyze the correlation between PRL-3 and E-cadherin expression with clinicopathological parameters. The correlation of PRL-3 and E-cadherin expression level with epithelial ovarian cancer was analyzed with Pearson's test. We used $p<0.05$ as statistically significant. Data were analyzed using SPSS for windows version 20.

RESULTS

We included 40 women who experienced epithelial ovarian cancer. The youngest age was 24 and 59 years old for the oldest patients. Most of the patients were between 31-40 years old (35%) and 41-50 years old (35%). Most of them were jobless (65%) and they had 1 to 4 children. In this study, 15 women were at early stage (37.5%) and 25 women (62.5%) were at advanced stage of the disease. Poor differentiation degree was mostly found in 47.5% patients (Table 1).

Table 1. Subject Characteristics

Characteristics	n	%
Age (years old)		
≤ 30	2	5.0
31-40	14	35.0
41-50	14	35.0
>60	10	25.0
Occupation		
Jobless	26	65.0
Governmental officer	7	17.5
Private officer	7	17.5
Parity		
Nulliparous	13	32.5
1-4	21	52.5
≥5	6	15.0
Stages		
Early	15	37.5
Advanced	25	62.5
Differentiation degree		
Good	11	27.5
Mild	10	25.0
Poor	9	47.5

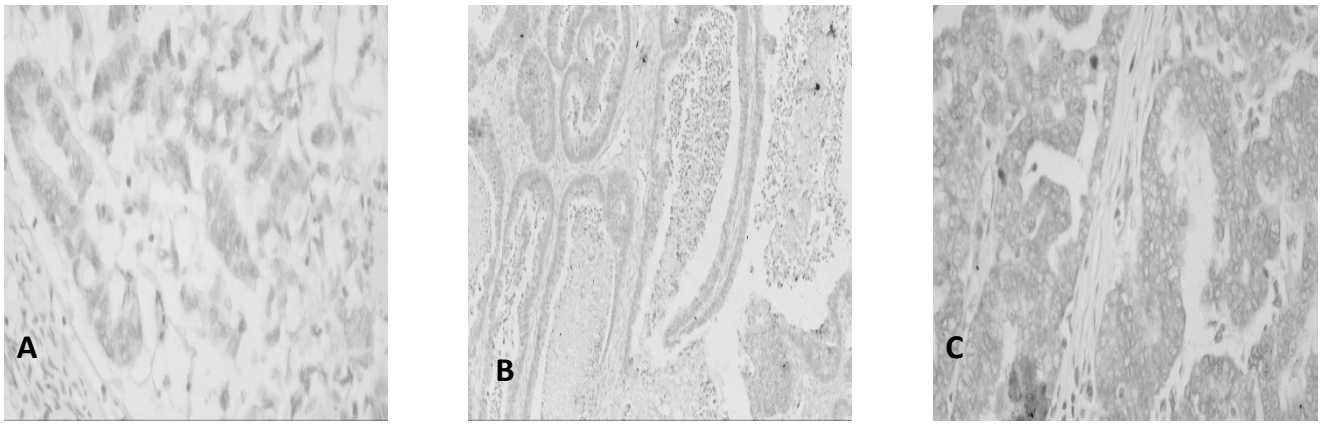

Figure 1. Immunohistochemical Staining of PRL-3 Expression in Epithelial Ovarian Cancer.
A = Grade I, **B** = Grade II, **C** = Grade III.

Figure 1 showed the expression of PRL-3 in epithelial ovarian cancer using immunohistochemical staining. The PRL-3 expression at various stages and degrees of differentiation showed no significant difference between the expression of PRL-3 at early stage compared to advanced stage. Apart from that, it pointed out the similar result to various degrees of epithelial ovarian cancer differentiation (p> 0.05) (Table 2).

The expression of E-cadherin in epithelial ovarian cancer using immunohistochemical staining was showed in Figure 2. E-cadherin expression at various stages and degrees of differentiation was found significantly different. High expression of E-cadherin was observed in early stage; while, low expression was seen in advanced stage (p<0.05). Conversely, there was no significant difference found between E-cadherin expression and degree of differentiation (p>0.05) (Table 2).

Table 3. Correlation PRL-3 and E-cadherin

	E-cadherin
PRL-3	rs = 0.161*
	p = 0.320*

**Spearman correlation test*

The statistical analysis showed no significant association between the expression of PRL-3 and E-cadherin in epithelial ovarian cancer (p>0.05). Therefore, it could be inferred that the expression of PRL-3 was not associated with the decreased expression (down-regulation) of E-cadherin in epithelial ovarian cancer.

DISCUSSION

This study showed the PRL-3 expression on the primary tumor tissue from 40 samples of epithelial ovarian cancer using the immunohistochemical

Table 2. PRL-3 and E-cadherin Expression on Stage and Differentiation Degree of Epithelial Ovarian Cancer

Variables	PRL-3		*p-value**	E-cadherin		*p-value**
	Low	High		Low	High	
Stage						
Early	13 (86.7)	2 (13.3)	1.000	2 (13.3)	13 (86.7)	<0.001
Advanced	21 (84.0)	4 (16.0)		19 (76.0)	6 (24.0)	
Differentiation degree						
Good	9 (81.8)	2 (18.2)	1.000	5 (45.5)	6 (54.5)	0.727
Poor	25 (86.2)	4 (13.8)		16 (55.2)	13 (44.8)	

**Fisher's Exact*

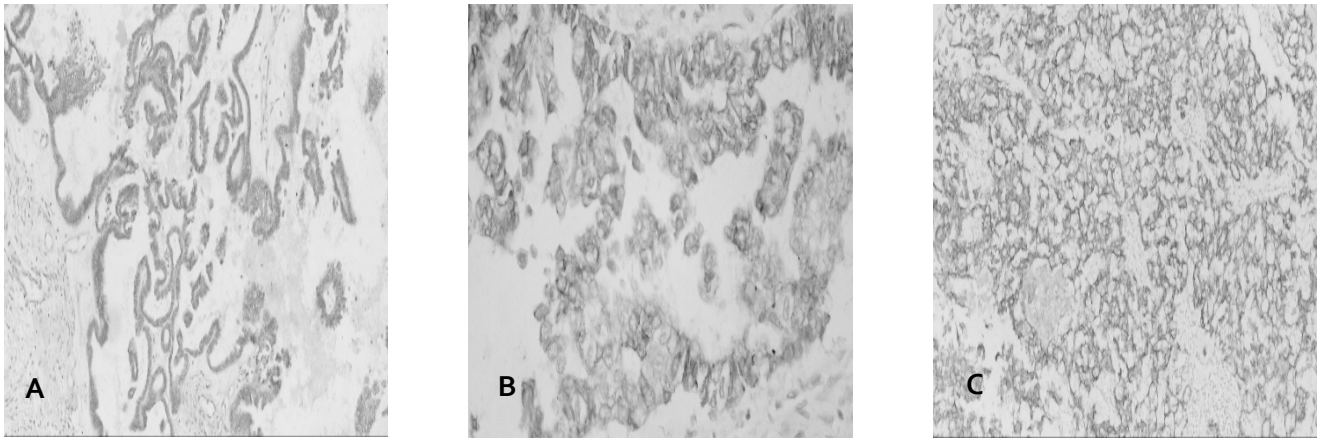

Figure 2. Immunohistochemical Staining of E-cadherin Expression in Epithelial Ovarian Cancer.
A = Grade I, **B** = Grade II, **C** = Grade III.

method. Positive expression of PRL-3 was found in 34 samples of primary ovarian tumors. This study found no significant association between PRL-3 expression in primary tumor and epithelial ovarian cancer stage. The similar result reported by Reich, et al. stated that there was no significant difference between the expression of PRL-3 and ovarian cancer stage.[9] In contrast, Polato, et al. found PRL-3 expression was high at an advanced stage than early stage.[10] It could be due to the sample size which involved 61 samples in stage I and 23 samples in stage III. Meanwhile, Reich, et al. study indicated fewer sample for early stage than advanced stage.

The PRL-3 expression was not significantly different in degrees of differentiation. Similar finding was reported by Reich, et al. and Polato, et al. studies which stated that there was no association between the expression of PRL-3 and degree of differentiation. Interestingly, Liu, et al. found up-regulation of PRL-3 ovarian cancer only occurred in differentiated cancer cells.[11]

E-cadherin plays a role in maintaining cell adhesion to prevent tumor cell invasion. E-cadherin mediated intercelullar adhesion that connects to cytoskeleton and cathenin as an intracellular binding protein. Low expression of E-cadherin will lead to detach tumor cells and attach to the matrix component resulting in migration and metastasis. E-cadherin tumor suppression gene has been widely studied in tumorigenesis. The interaction between E-cadherin molecules is crucial to the formation of adhesion among cells and maintain the cells adhesion. The loss of E-cadherin is associated with the transition from benign lesions to the invasive and metastatic lesions.[12]

In this study, the protein E-cadherin was expressed in all primary epithelial ovarian cancer. Although, there was no significant difference of E-cadherin expression in various degrees of differentiation, there were statistically significant difference of the E-cadherin expression in epithelial ovarian cancer stage. High expression of E-cadherin was found in early stage than advanced stage. Study by Faleiro-Rodrigues, et al. revealed a significant decreased expression of E-cadherin based on FIGO staging and they showed no correlation to tumor differentiation.[13] In another study on the expression of E-cadherin and its relationship with ovarian cancer showed that a decreased expression of E-cadherin was associated with the severity of ovarian cancer based on FIGO staging, lymph node metastasis, and degree of tumor differentiation. This result would like to reveal that the expression of E-cadherin tended to decrease with the increasing of stage disease and poorly differentiated cancer cells.[14]

This is the first study that reported association between the expression of PRL-3 and E-cadherin. We revealed that the increased levels of PRL-3 did not affect the levels of E-cadherin and also vice versa. Our result was contrary with the result by Liu, et al. Liu found that the PRL-3 increased the epithelial mesenchymal transition (EMT) by down-regulated expression of E-cadherin. The EMT enhancement mechanism caused by PRL-3 was still unknown. Liu, et al. found that PRL-3 might improve the EMT in colorectal cancer cells SW480 models deficient in the expression of E-cadherin in vivo and in vitro. The overexpression of PRL-3 or SW480 cell solution was injected subcutaneously in nude mice. The result of immunohistochemical analysis of tumor samples from nude mice showed that the PRL-3 increased the up-regulation of mesenchymal marker vimentin and down-regulation epithelial marker of E-cadherin and cytokeratin.[11] Guo, et al. study on the ability of PRL-3 triggering tumor angiogenesis reported that subcutaneous injection of PRL-3 in nude mice led to increase formation of endothelial cells in the tumor mass.[5] In contrast, Sundfeldt, et al. revealed that the down-regulation or abnormal expression of E-cadherin was associated with highly invasive capacity and poorly differentiated epithelial ovarian carcinoma.[6]

CONCLUSION

Our study has shown that E-cadherin plays an important role in the progression of epithelial ovarian cancer. This suggests that E-cadherin might have therapeutic value and a potential biomarker in the progression of ovarian cancer. The PRL-3 and E-cadherin also plays a role in ovarian cancer metastasis. Therefore, we need to investigate the role of PRL-3 and E-cadherin in the process of ovarian cancer metastases for future studies.

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